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ALPHA 1 - ANTITRYPSIN: A FUTURE ROLE IN THE PREVENTION OF PANCREAS GRAFT REJECTION AND TREATMENT OF TYPE 1 DIABETES

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ABSTRACT

In 2016, the World Health Organization (WHO) issued a global report on diabetes indicating that almost 422 million people worldwide suffer from some form of diabetes. It is estimated that between 5% and 10% of those number suffer from Type 1 diabetes mellitus, which is associated with self-recognizing antibodies leading to the destruction of pancreatic islet cells, insufficient insulin production and insulin-sensitive hyperglycemia. It is estimated that approximately 80,000 children are diagnosed with the disease each year, and the WHO projects that diabetes will be the 7th leading cause of death by 2030. Increasingly, pancreas transplantation has become a viable option for controlling blood glucose levels and restoring islet function in patients. The transplantation of pancreatic insulin producing islet cells has risen in prevalence as a less invasive alternative to whole organ transplantation in patients. However, graft rejection is a major issue for pancreas transplantation where 80% of islet grafts survive for 1000 days, and only 13% of islet grafts survive for 5 years.

Currently, patients receiving pancreatic grafts take immunosuppressive drugs which prolong graft survival while increasing the host's risk for infection. Successful protection of pancreatic grafts requires proper management of maintenance therapy to reduce the activity of autoreactive immune cells causing islet cell destruction and to reduce inflammatory reactions to the transplanted graft.

Alpha 1-antitrypsin (AAT) is a serine protease primarily produced by hepatocytes to limit local inflammation and is also known to be expressed by primary phagocytes, islet cells, neutrophils and epithelial cells. AAT is currently in use as an orphan drug approved for treatment of alpha 1-antitrypsin deficiency and is undergoing promising clinical trials for management of type 1 diabetes.

This presentation explores the mechanisms behind the anti-inflammatory properties of alpha 1-antitrypsin, and proposes that alpha 1-antitrypsin has promising applications as an immunomodulator for the protection of pancreatic cells, improvement of islet function and reduction of pancreatic graft rejection.

KEYWORDS: Diabetes, Type 1, Alpha 1-Antitrypsin, AAT, Islet Cells, Pancreas, Graft Rejection & Transplants

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Original Article

INTRODUCTION

The American Diabetes Association reports that approximately 1.4 million Americans are diagnosed with diabetes each year (ADA website, 2016). Type 1 diabetes mellitus is associated with self-recognizing antibodies leading to the destruction of pancreatic islet cells, insufficient insulin production and insulin-sensitive hyperglycemia (Delli & Lernmark, 2016). Pancreas transplantation has arisen as an option for controlling blood glucose levels and restoring islet function in patients (Frank et al., 2004). The transplantation of pancreatic insulin producing islet cells has risen in prevalence as a less invasive alternative to whole organ transplantation in patients

(Frank et al., 2004). Graft rejection is a major issue for pancreas transplantation. 80% of islet grafts survive for 1000 days; however, after this period of time the survival rates of pancreatic grafts falls rapidly until only 13% of islet grafts survive for 5 years (Berman et al., 2009).

One of the key challenges to reducing pancreas graft rejection is proper management of the host's immune response to prevent continued destruction of islet cells. Graft rejection has been shown to correlate with an alloimmune response due to over activity of T-cells and production of anti-MHC molecules (Naftanel and Harlan, 2004). Currently, patients receiving pancreatic grafts take immunosuppressive drugs which prolong graft survival while increasing the host's risk for infection (Male et al., 2013). Successful protection of pancreatic grafts requires maintenance therapy to reduce the activity of autoreactive immune cells causing islet cell destruction and to reduce inflammatory reactions to the transplanted graft.

Alpha 1-antitrypsin (AAT) is a serine protease primarily produced by hepatocytes to limit local inflammation and is also known to be expressed by primary phagocytes, islet cells, neutrophils and epithelial cells (Ehlers, 2014). AAT is currently in use as an orphan drug approved for treatment of alpha 1-antitrypsin deficiency and is undergoing promising clinical trials for management of type 1 diabetes (Lewis et al., 2016). This manuscript explores the mechanisms behind the anti-inflammatory properties of alpha 1-antitrypsin, and proposes that alpha 1-antitrypsin has promising applications as an immunomodulator for the protection of pancreatic cells, improvement of islet function and reduction of pancreatic graft rejection.

DISCUSSIONS

Alpha 1-Antitrypsin Affects Humoral and Cell-Mediated Immunity

Alpha 1-antitrypsin (AAT) has been indicated to produce anti-inflammatory effects by decreasing the release of cytokine TNF- α, which promotes the immune response through recruitment of neutrophils and eosinophils and activation of the MAPK pathway, and IFN-ɣ, secreted by immune cells to provoke a response to viral and microbial infections (Yang et al., 2015). In addition to neutrophils, eosinophils and macrophages, the immune system relies on a population of T and B lymphocytes to protect the body from infection and foreign materials (Male et al., 2013). The reduction of inflammatory response observed by exposure to AAT may occur through interference with B or T lymphocytes. Lipopolysaccharide (LPS) stimulated cultures of B-lymphocytes incubated with h-AAT increased production of IL-10 by 1.5 ± 0.03 fold when compared to LPS stimulated control cultures (Mizrahi et al., 2013). IL-10 is an anti-inflammatory cytokine produced by a wide population of immune cells to promote differentiation of regulatory T cells (T_{reg}), to suppress cytokine synthesis and to reduce the inflammation causing actions of natural killer cells, macrophages and T_h 1 helper cells. (Couper, Blount & Riley, 2008; Male, Bronstoff, Roth & Roitt, 2013). IL-10 downregulates the activity of T_h 1 cells by limiting production of IL-12 and IFN-ɣ by antigen presenting cells (Male et al., 2013). An AAT-dependent increase in IL-10 secretion would explain the observed decrease in IFN-ɣ and inflammation.

Most research has focused on h-AAT's effect on helper and regulatory CD4+ T-cells, while the response, of B-lymphocytes to incubation with and/or transgenic expression of h-AAT, suggests that h-AAT affects both humoral and cell-mediated immunity. In addition to affecting the activity of T cells, incubation with AAT also may affect antibody release from B lymphocytes. Transgenic h-AAT mice receiving allogeneic skin grafts displayed a decrease in IgG antibody, suggesting less inflammation and/or a reduced adaptive immune response, and splenic lymphocytes incubated *in vitro* with h-AAT (0.5 mg/mL) displayed a decrease in IgM antibody release in the presence of inflammation inducing LPS

(Mizrahi et al, 2013). The noticed decrease in IgM and IgG antibody demonstrates a suppression of immune response and B cell antibody production in response to AAT.

The same protective qualities of AAT which protect islet cells from immune cells have also been shown in murine models to promote and enhance pancreatic graft survival and function (Wang, Yan, Zhou & Li, 2014). Administration of alpha 1-antitrypsin as an anti-inflammatory should be considered in immunosuppression regimes during pancreas transplantation in order to reduce graft rejections due to inflammatory events. Mice receiving serial doses of h-AAT for thirty days achieved pancreatic graft transplantation acceptance and normoglycemia (Lewis et al., 2016). Cessation of h-AAT treatment after thirty days resulted in continued function of graft islets. This information suggests that h-AAT treatment during the initial days after transplantation may significantly increase host tolerance to the donor graft. Undifferentiated T-cells lacking either CD4 or CD8 markers were found surrounding the areas of transplantation in this study (Lewis et al., 2016).. Pancreatic grafts incubated with h-AAT in mice demonstrated increased level of CTLA-4, Foxp3 and TGF-β, gene expression correlated to regulatory T cells, suggesting that- h-AAT affects the immune system through promotion of regulatory T cell differentiation (Lewis et al., 2016). Fluorescent microscopy showed increased infiltration of Foxp3 expressing cells, presumed to be regulatory T cells, in transplanted grafts (Lewis et al., 2016).

T cells show a great deal of plasticity, and $FoxP3^{+}$regulatory T cells are capable of altering phenotype in order to simultaneously express inflammatory and anti-inflammatory molecules (Male et al., 2013).These findings suggest a role for h-AAT in not disabling the immune system and development of lymphocytes but in promoting differentiation of non-inflammatory T cells.

Insulin producing murine NT-1 cells used to model pancreatic β cells transfected to express human alpha 1-antitrypsin displayed increased levels of IL-4 and decreased levels of IFN-ɣ in the supernatant of lymphocytes (Wang et.al, 2014). IL-4 inhibits production of IFN-ɣ, an inflammatory molecule and promotes increased differentiation of T_h 2 helper T cells from the T_h 0 population, thus shifting the immune system away from T_h 1 helper T cells (Wang et.al, 2014). These findings are supported by a study administering human AAT to non-obese diabetic mice, which found decreased expression of T_h 1 specific transcription factors, suggesting a decrease in T_h 1 cells (Koulmanda et al., 2008). IL-4 is a B-cell growth factor produced by T-cells which activates B lymphocytes and increases differentiation of T_h 2 helper T cells to increase IgG_1 and IgE expression (Male et al., 2013). Implementing an immunosuppressant regime which, instead of crippling the patient's immune response, thereby increasing risk for concurrent infections, would adjust the ratio of T_h 1 to T_h 2 cells, may serve to sufficiently decrease the risk of autoimmune graft rejection while allowing for a healthy immune system.

Alpha 1-Antitrypsin Decreases Expression of MHC Class II Molecules

MHC II molecules are expressed by immune cells for displaying antigens to immune cells and activating the immune response while MHC class I molecules are expressed on non-immune cell surfaces (Male et al., 2013). A clinical study on patients receiving kidney and simultaneous kidney-pancreas transplantation demonstrated a correlation between allo-antibodies reacting to MHC class II molecules and acute graft rejection (Pelletier, Hennesy, Adams, VanBuskirk, Ferguson &, Orosz, 2002), but not MHC class I molecules. These findings suggest that antibody binding, to MHC class II molecules, are more responsible for acute rejection of pancreatic transplants than MHC class 1 molecules. The risk for patients to develop chronic graft rejection was deemed to increase in patients with a history of acute rejection and production of MHC reactive antibodies (Pelletier et al, 2002). Further statistical analysis showed that the presence of

anti-MHC class II molecules predicted chronic graft rejection independent of the development of acute graft rejection (Pelletier et al, 2002). High levels of anti-MHC class I antibody have been observed in patients experiencing acute transplantation rejection, but this study suggests that anti-MHC II antibodies are greater predictors of both chronic and acute graft rejection. It also suggests that presentation of donor antigen by MHC II molecules plays a greater role in graft rejection.

At a hospital in Geneva, Switzerland, patients receiving whole organ pancreas transplants and islet transplantation were assessed for antibodies recognizing HLA, the human equivalent of MHC. Mean fluorescence intensity (MFI) was used to determine whether the presence of anti-HLA was correlated with graft rejection and hypersensitivity. Donor specific antibodies were produced in the majority (61.1%) of patients receiving islet transplants and in many (46.2%) patients with pancreas transplantation (Chaigne et al., 2016). The level of match of HLA, for either class I or class II, between donor and recipient did not influence the development of donor specific anti-HLA antibodies after pancreatic or islet transplantation or affect graft survival (Chaigne et al., 2016). These findings contradict each other; warranting further research on the role of MHC class II antibodies and acute graft rejection and whether islet or whole pancreas transplantation plays a critical factor.

It is likely that MHC class II alloantibodies do affect graft survival as transgenic h-AAT mice showed a decrease of surface MHC II expression in B-lymphocytes in response to stimulation with LPS (Mizrahi et al., 2013). A low MHC II density favors differentiation of $T_h 2$ helper cells (Male et al., 2013). Therefore, h-AAT's effect on MHC II expression may alter the balance of $T_h 1$ / $T_h 2$ helper T cells engaged in cell-mediated immunity, instead of altering the development of MHC II antibodies after transplantation.

Alpha 1-Antitrypsin Decreases Granulation of Natural Killer Cells

AAT has been shown to affect expression of HLA and MHC molecules and affect the differentiation of T cells however; natural killer (NK) cells have been known to play a role in autoimmune attacks and promote cell lysis; therefore, they may be a contributing factor to the destruction of pancreatic islet cells (Male et al., 2013). Insulin producing islet cells contain ligands recognized by at least two different types of receptors present on NK cells (Guttman, Yossef, Freixo-Lima, Rider, Porgador, & Lewis, 2014) that lead to destruction of insulin producing pancreatic cells and ultimately development of hyperglycemia and type 1 diabetes. One study questioned whether AAT plays any role in the immunomodulation of natural killer (NK) cell activity. In murine bone marrow dendritic cell cultures, incubation with AAT decreased degranulation upon stimulation with IFN-ɣ (Guttman et al., 2014). Exposure to AAT l reduced NK degranulation only when NK cells were stimulated with IFN-ɣ administration or through co-cultures with murine pancreatic transplant cells, but had no effects when applied directly to NK cells (Guttman et al., 2014). This suggests that the immunosuppressive effects of AAT are dependent upon inflammatory stimulation and do not possess any inherent effects on NK cells.

Fortuitously, although AAT exhibits an immunosuppressive effect on NK cells with regards to recognizing and attacking beta islet cells, incubation with AAT does not appear to inhibit NK response to other types of cells. Upon exposure to melanoma and insulinoma tumor cells, NK cells treated with AAT expressed increased levels of CD107 and activation comparable to controls (Guttman et al., 2014). One might expect the protective effects of AAT to extend to insulinomas of a beta cell line due to similar functions; but the cytotoxic effects of the NK cells to cancerous cells appears to be unhindered by exposure to AAT. If AAT treatment were to be administered as part of an immunosuppressive therapy for type 1 diabetes or pancreatic graft transplantations, the ability of NK cells to respond to cancerous cells may remain.

These findings suggest that AAT plays a role in decreasing the cytotoxicity of NK cells, and this suppression of NK activity may be one of the mechanisms behind the ability of AAT to protect beta islet cells from autoimmune attacks due to type 1diabetes or as in response to allogeneic transplantation.

Alpha 1-Antitrypsin Affects Toll-Like Receptors

Toll-like receptors (TLR) are transmembrane receptors involved in T cell independent innate immune system, recognizing danger associated molecular patterns (DAMPs) and lipopolysaccharide (LPS) and is suspected to play a role in the development of type 1 diabetes (Giovannoni et al., 2014). LPS is a T-cell independent (TI) antigen capable of activating B lymphocytes without recognition from MHC II molecules (Male et al., 2013). TI antigens are capable of causing polyclonal B cell activation and may bind to Toll-like receptors (Male et al., 2013). This results in the ability of cytokine and antibody release without prior exposure to the antigen or in the proposed case, a pancreas graft (Male et al., 2013). The immune response to TI antigens occurs early and produces primarily IgM antibodies, suggesting a moderation of acute and hyper-acute graft rejection, but not chronic.

Anti-TLR4 monoclonal antibodies were used to reduce LPS signaling through Toll-like receptor 4. Incubating human islet cells with anti-TLR4 significantly decreased LPS-induced beta cell apoptosis compared to control islet cells ($P<0.05$). These findings suggest that TLR4 signaling contributes to beta cell apoptosis during autoimmune attacks and that reduction of TLR4 signaling may become a useful method of preserving beta cell survival. 100% of TLR4-mAb treated mice transplanted with anti-TLR4 mAb treated islets which did not suffer allorejection exhibited reversed diabetes as opposed to 75% from controls (Giovannoni et al., 2014). Additionally, anti-TLR4 treatment led to graft survival lasting longer than 100 days in 62.5% of mice. Compared to a median of 41.5 days when either only the islets or mice were treated with anti-TLR4 (Giovannoni et al., 2014). Control mice not receiving anti-TLR4 suffered graft rejection in a median of 12.5 days (Giovannoni et al., 2014). These findings show that blocking the TLR4 pathway prolongs the survival of islet grafts, and that double incubation of both graft and animal with an antagonist for TLR4 produces an optimum effect on graph survival.

AAT decreases the effect of LPS-induced TLR4 signaling by decreasing the LPS induced release of TNFα and IL-1β. In a clinical trial of humans diagnosed within four years with type 1 diabetes, subjects were administered with 80 mg/kg h-AAT and had measurements were taken of the fluorescent intensity of IL-1β expression in peripheral blood monocytes (Gottlieb ,Alkanani & Michels, 2014). IL-1β is linked to the development of type 1 diabetes in humans and correlated with dysregulation of toll-like receptors (Gottlieb et al., 2014). Twelve months after h-AAT therapy, the cellular level of IL-1β in human blood monocytes activated with either LPS or R848 monocytes, (ligands for TLR4 and TLR7 and 8 respectively) showed a significant decrease ($P<0.05$) in expression as measured by MFI from 16 to 6 (medium fluorescent intensity)(Gottlieb et al., 2014).

While IL-1β cell expression levels decreased at 12 months, levels began to rise at 18 months, suggesting that h-AAT therapy may not cause any permanent changes and treatments may require regular administration to provide anti-inflammatory and islet cell protecting effects (Gottlieb et al., 2014). The study observed a correlation between improved islet function in patients in response to administration of h-AAT and a decreased frequency of IL-1β expressing monocytes and dendritic cells in blood samples incubated with h-AAT (Gottlieb et al, 2014). Exposure to h-AAT reduces the expression of IL-1β, associated with dysregulated TLRs and improves islet function as measured by improved and stabilized C-peptide levels.

An increase in TNF-α release from mixed AAT and LPS exposure was successfully inhibited by anti-CDL14 antibodies when incubation was for 2 hours, but anti-CDL14 antibodies exhibited no effect on cells cultured for 18 hours (Nita et. al, 2007). Since CD14 lacks a transmembrane domain, it is thought to bind LPS and present the ligand to toll-like receptors such as TLR4. These findings suggest that AAT may in fact be recognized by CD14 and not directly by TLR4. LPS interacts with a combination of TLR4 and CDL14 receptors to activate the NF-κB pathway which leads to a chain of events promoting inflammatory molecule production (Nita, Serapinas, & Janciauskiene, 2007). Previous studies have shown h-AA reduces the release of molecules from LPS-mediated signaling through disruption of TLR4 however; LPS-stimulated cells incubated with AAT displayed decreased levels of both CD14 and TLR4 mRNA and protein expression (Nita et al., 2007). These findings suggest that AAT depresses inflammatory immune responses through not solely the CD14 or TLR4 pathway, but both.

Simultaneous two hour long exposure of LPS and AAT to human monocytes generates an increase ($p<0.001$) in TNF-α, IL-8 and IL-1β, molecules moderating inflammation and immune response. These findings seemingly contradict later findings of reduced IL-1β, secretion in response to h-AAT incubation (Gotlieb et al, 2014); however, a longer 18 hour culture with LPS and AAT inhibited the expression of these molecules and support an observation of AAT induced reduction of IL-1β secretion. (Nita et al., 2007). AAT produces ultimately anti-inflammatory effects through a time-dependent mechanism and are contrary to later study which failed to find altered expression of TNF- α gene expression in pancreatic lymph nodes (Koulmanda et al., 2008). The short-term increase in inflammatory molecules may explain the observation of anaphylactic shock in rodents after the fourth injection of human derived AAT in non-obese diabetic mice used as a model for type 1 diabetes (Lu et al., 2008).

Another possible explanation is that AAT reduces of TNF-α induced activation of the NF-κB pathway. A study using flow cytometry and Western blot analysis found that human cells incubated with physiological levels of AAT reduced TNF-α binding to both TNFR1 and TNFR2 receptors, and reduced degradation of the antagonist NF-κBα (Bergin et al., 2004). Increased presence of an antagonist to the NF-κB pathway will ultimately reduce levels of NF-κB pathway dependent inflammation from TNF-α release.

CONCLUSIONS

This manuscript explored the mechanism behind alpha 1-antitrypsin induced immunosuppression and proposes that the anti-inflammatory properties of alpha 1-antitrypsin have promising applications as an immunomodulator for protection of pancreatic cells, improvement of islet function and to reduce rejection of islet grafts. The mechanism of AAT modulation of the inflammatory response may occur through promoting the differentiation of regulatory T cells and adjusting the balance between T_h1 and T_h2 helper T cells to increase T_h2 differentiation. In addition, AAT reduces MHC class II expression on the surface of B lymphocytes. Although studies have been controversial over whether antibodies recognizing MHC class II proteins after pancreas transplantation affect graft survival, reduced MHC class II expression may suppress inflammatory reactions by promoting T_h2 differentiation. AAT has also been observed to promote graft survival and protection by reducing TLR4 signaling response to LPS. These findings suggest a wider available use for this orphan drug in the treatment of patients suffering from type 1diabetes by protecting beta cell islet function and increasing the length of survival for pancreatic grafts. Concerns over the anaphylactic reaction of non-obese diabetic mice given repeated doses of h-AAT warrants caution; however, but may be a function of h-AAT's time dependent mechanism of function. Treatments of h-AAT should be temporally spaced so that the initial AAT dependent increase in inflammatory

factors does not compound; however promoted T_h2 differentiation should be monitored closely as type 2 helper T cells mediate allergic reactions and have been implicated to play a role in acute hypersensitivity. (Male et al., 2014) Although recent findings on the use of alpha 1-antitrypsin to prolong pancreatic graft survival and manage type 1 diabetes have been promising, there are limitations which warrant further research.

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